# Cryostructuration of Polymer Systems. XXIV. Poly(vinyl alcohol) Cryogels Filled with Particles of a Strong Anion Exchanger: Properties of the Composite Materials and Potential Applications

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**ABSTRACT:** A composite material produced from a poly(vinyl alcohol) cryogel with entrapped particles of the strong anion-exchange resin Amberlite is presented. The properties of the composite material depended strongly on whether the resin was used in $OH^-$ form or $Cl^-$ form. The ion-exchange filler in $OH^-$ form caused both a significant reinforcement of the composite material and an increase in the gel fusion temperature. These effects were thought to be associated with the additional ionic bonding between the continuous and disperse phases. Beads 200–600 $\mu$m in size were prepared from the composite material and used in expanded-bed ion-exchange chromatography for the capture of the negatively charged solutes benzoate and lactate from the suspension of negatively charged cells. The plausibility of the approach has been demonstrated on model systems composed of yeast cells and benzoate and with a real fermentation broth produced after lactic acid fermentation.
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**Key words:** gelation; ion exchangers; composites

## INTRODUCTION

Cryotropic treatment (single or repeated cycles of freezing–thawing) of concentrated solutions of poly(vinyl alcohol) (PVA), which has a high degree of saponification (a small content of residual nonhydrolyzed O-acyl groups), is known to produce macroporous gels, so-called poly(vinyl alcohol) cryogels (cryoPVAGs).[1–4] These gels are of interest as materials for different applications, particularly, in biotechnology and biomedicine.[1–8]

In general, the latter materials are composed not only of PVA and a solvent but also contain some other components such as soluble additives (e.g., electrolytes, carbohydrates, synthetic polymers) and/or insoluble disperse particles (fillers). All of the main factors that affect the cryoPVAG properties (e.g., PVA characteristics, conditions of freeze–thaw treatment) have a similar effect on the properties of composite cryoPVAGs.[9–12] In addition, the nature (e.g., surface hydrophilic–hydrophobic properties) and amount of filler particles and their mechanical properties, size, shape, and structure (e.g., porosity, which determines the penetration of PVA macromolecules inside the filler particles) influence the physicomechanical and thermal characteristics of filled poly(vinyl alcohol) cryogels (f-cryoPVAGs), especially on the cryogel microstructure at the interface of the continuous (PVA gel) and dispersed (filler) phases.[9,10]

However, the mechanisms of the filler effects on the properties of f-cryoPVAGs remain mainly unexplored. Thus, it is known that soluble electrolytes affect the formation of cryoPVAGs and their physical properties,[13–15] but the effect of additives with covalently bound ion-exchange groups on cryoPVAG matrix has not been studied.

Our interest in composite cryoPVAGs containing ion-exchange resin was also due to the potential application of these materials in bioseparation. The production of water-soluble chemical substances by fermentation presents increasingly an attractive alternative to traditional chemical synthesis. As a rule, the

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raw material resulting after fermentation is a rather dilute aqueous solution of the target product containing numerous cells of the microorganism producer. The first stage of the purification of nonvolatile chemicals from dilute aqueous solutions is a product capture by adsorption to an insoluble adsorbent. The product is then eluted as a much more concentrated solution and subjected to further purification. The use of expanded-bed adsorption allows for direct processing of cell-containing fermentation liquids in a chromatographic mode.[16] However, the direct capture of anionic product from the fermentation broth with anion exchanger is hindered by the presence of cells. As microbial cells are negatively charged, they tend to adsorb to the anion exchanger, compromising the stability of the expanded bed and, finally, clogging the system.[17]

The entrapment of small particles of anion exchanger into a macroporous gel will result in the sterical protection of the exchanger and, thus, solves this problem. CryoPVAGs have a system of large (0.1–1.0 $\mu$m) interconnected pores. The pore size depends on the initial polymer concentration and the freeze–thaw conditions.[3,7,18–20] The pores are too small for microbial cells to enter the particles of the cryoPVAGs and interact directly with the ion exchanger, whereas small solutes can penetrate inside the composite material and be absorbed by the ion-exchange resin with no difficulties.

The objective of this study was to examine how the entrapment of additives with anion-exchange groups affects the properties of composite cryoPVAGs and to evaluate the potential of the produced composite materials for the direct capture of anionic solutes from the fermentation broth.

## EXPERIMENTAL

### Materials

#### Chemicals

Atactic PVA, 99% hydrolyzed with a molecular weight (MW) of 89–98 kDa (Aldrich Chemical Co., Milwaukee, WI), was used without additional purification. Amberlite IRA-410 resin was purchased from Serva (Heidelberg, Germany). Epichlorohydrine, benzoic acid (BA), and sodium salt of lactic acid (LA) were from Sigma (St. Louis, MI). Organic Acid Standard was obtained from Bio-Rad (Hercules, CA). The others reagents were the best quality available.

#### Microorganisms and culture medium

The yeast, *Saccharomyces cerevisiae*, was obtained from a local supermarket. The cells of *Lactobacillus delbrueckii*, subspecies *bulgaricus* (DSM 20081), were cultivated in a 250-mL flask containing 100 mL of sterile synthetic medium, which included 15 g of potassium dihydrogen phosphate, 15 g of dipotassium hydrogen phosphate, 10 g of sugars (lactose:glucose = 1:19), 0.005 g of magnesium sulfate heptahydrate, 0.0031 g of manganese sulfate monohydrate, 0.002 g of ferrous sulfate monohydrate, 0.005 g of ascorbic acid, and 10 g of yeast extract at pH 6. The cells were incubated for 48 h at 40°C in an anaerobic jar. LA production was confirmed by High Performance Liquid Chromatography (HPLC) analysis.

### Methods

#### Preparation of the filler

Commercial Amberlite resin was ground in mortar, and a fraction of the particles 25–100 $\mu$m in size was separated with calibrated sieves. The filler particles were equilibrated with water and pretreated with an excess of 0.5*N* NaOH or 0.5*N* HCl to obtain the anion-exchange resin in $OH^-$ or $Cl^-$ forms, respectively. Finally, the resin was rinsed with water until the pH was around 7.0. The excess of water was then removed by vacuum filtration on a porous glass filter. The pretreated filler was stored in a desiccator at 100% humidity.

#### Preparation of the filler suspension in a PVA solution

A defined amount of PVA was suspended in the required volume of deionized water. The mixture was stored at room temperature overnight for the polymer to swell. All of the PVA dissolved when the suspension was heated for 30 min under stirring in a boiling water bath. The sample was weighed before and after heating, and the amount of evaporated water was compensated. An appropriate amount of filler was suspended in the PVA solution and allowed to stay at room temperature for 21 h for equilibration. Then suspension was mixed again and heated in a boiling water bath for 10 min; the amount of evaporated water was compensated. As the PVA concentration in the solution changed after mixing and equilibration with a water-swollen filler as the function of filler content, the calibration curve for the dependence of PVA concentration in solution on the amount of swollen filler added was obtained. The polymer concentration in the continuous phase of each sample was 100 $\pm$ 1 g/L.

#### Formation of cryogel blocks

Blocks of nonfilled cryoPVAGs and f-cryoPVAGs for the study of mechanical properties were formed in sectional duralumin molds (inner diameter = 15 mm, height = 10 mm). For the determination of fusion temperature ($T_f$), cryogels were prepared in transpar-

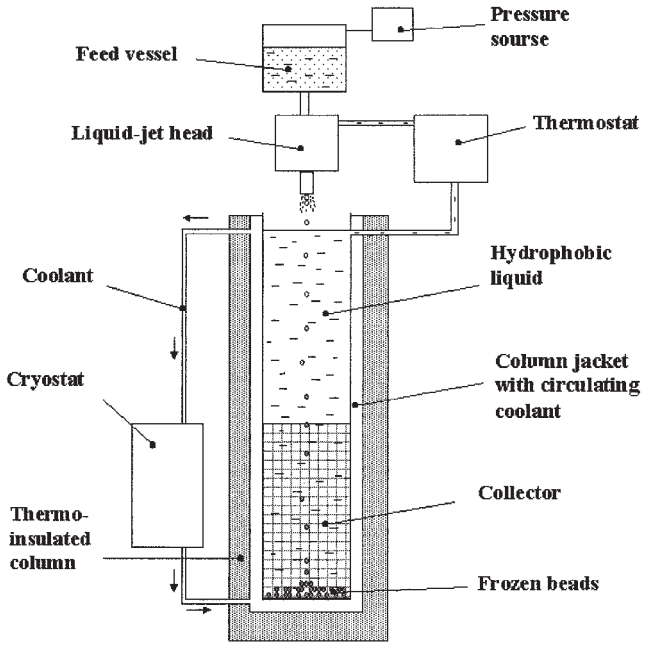



**Figure 1** Cryogranulation setup for the preparation of cryoPVAG and f-cryoPVAG beads.

ent polystyrene test tubes (inner diameter = 9 mm). A PVA solution or suspension with a filler was poured into each tube in the amount of 2 mL, and a metal bead (stainless steel; diameter = 3.5 mm, weight = 0.275 ± 0.005 g) was placed on the bottom. The tubes were plugged with a plastic screw top. The molds and tubes were placed into the chamber of a precision programmable cryostat (FP 45 HP, Julabo, Seelbach, Germany). The samples were frozen at −20°C for 5.5 h. Then, the temperature was raised to 20°C at a rate of 0.03°C/min, and the samples were stored at that temperature for at least 30 min before mechanical or thermal property determination.

### Formation of cryogel beads

The ground filler was mixed with PVA solution (100 g/L) to give a uniform 30% (w/v) suspension. The f-cryoPVAG beads were formed with a cryogranulation setup[21] (Fig. 1). The suspension of PVA with filler was pressed into a liquid-jet head, where the jet was splinted into droplets by the flow of a water-immiscible solvent (petroleum ether). The droplets of the suspension fell down into the column filled with the same solvent cooled to −20°C and were frozen to form spherical beads. The frozen beads were gathered in a collector at the bottom of the column. The beads were kept frozen at −20°C overnight and were then thawed at a rate of 0.008°C/min. After the thawed beads were washed with deionized water, they were crosslinked with epichlorohydrine (0.01% in 0.1*M* NaOH) under shaking on a rocking table for 4 h. Finally, the crosslinked f-cryoPVAG beads were washed with deionized water until the washing waters were neutral. The spherical beads obtained were 200−600 $\mu$m in size.

### Mechanical properties of nonfilled cryoPVAGs and f-cryoPVAGs

The mechanical properties of cryoPVAGs and f-cryoPVAGs were studied with the penetration method,[22] essentially in accordance with the procedure described elsewhere in detail for both nonfilled cryoPVAGs and f-cryoPVAGs.[9–13] During the measurements, the load was 9.8 mN, and the punch had a spherical top 5 mm in diameter. To prevent surface drying, the samples were covered with fine layer of silicon oil. The mechanical parameter calculated from the creep data was apparent instantaneous shear modulus ($G_0$). The $G_0$ values were further used to compare the properties of cryoPVAGs or f-cryoPVAGs. The modulus for filled cryogels is designated by the superscript *f*. When the results are discussed, shear modulus is presented as a ratio, $G_0^f/G_0$, for each sample of the composite to nonfilled cryogel with the same polymer concentration in the continuous phase and prepared under the same conditions.

### Determination of gel $T_f$'s

The $T_f$ values of cryoPVAGs and f-cryoPVAGs were determined as follows. Tightly plugged test tubes with cryogel and with a metal bead at the bottom were placed bottom up into a water bath with an agitator. The samples were heated at a rate of 0.4 ± 0.1°C/min. The temperature, when the metal ball passing through cryogel matrix fell onto the plug, was registered as $T_f$.

### Interaction of yeast cells with f-cryoPVAG and Amberlite resin

A suspension of cells (30 mL, 10 mg/mL) in water was passed through the column (inner diameter 10 mm) equilibrated with deionized water in an expanded-bed mode at linear flow rates of 260 and 764 cm/h for f-cryoPVAG and for Amberlite resin, respectively, to get the same twofold extension degree of the bed. The chromatographic experiments were carried out with a 200 mm long glass column with an inner diameter of 10 mm and fitted with two adapters (Amersham Biosciences, Uppsala, Sweden). The bottom adapter was fitted with a stainless steel mesh flow distributor. When the flow was directed upward, the chromatographic bed expanded after some critical flow rate was reached. The increased distance between the beads in the expanded bed allowed the cells to pass through the bed without being trapped mechanically, as in the packed-bed mode, which performed as a depth filter.

The elution of bound cells was performed with 0.5*M* NaOH in an upward mode at the same flow rate. The content of cells in the effluent was analyzed by measurement of the absorbance at 600 nm.

### Adsorption of BA by f-cryoPVAG and Amberlite resin

The adsorbent ($OH^-$ form, 1.5 mL) was suspended in 1.5 mL of deionized water and transferred into a flask containing a solution of BA (1 mg/mL) adjusted with NaOH to pH 7.0. The adsorption experiment was performed at room temperature and constant stirring. Samples (0.8 mL) were quickly removed from the flask at defined time intervals and centrifuged 3 min at 6000 $\times$ g. The absorbance of the supernatant was measured at 270 nm.

### Adsorption of BA by f-cryoPVAG beads in an expanded-bed mode

Beads of f-cryoPVAG (5-mL settled volume) were put into a glass column (inner diameter = 10 mm) and washed in an expanded-bed mode at a linear flow rate of 260 cm/h with 10 bed volumes of 0.5*M* NaOH followed by deionized water until the pH was close to 7.0. A solution of BA (2 mg/mL, adjusted to pH 7.0) with different contents of yeast cells (0, 10, 20, and 40 mg/mL) was applied to the column in an expanded-bed mode at the same linear flow rate. The column was washed with deionized water, also in an expanded-bed mode. The flow was interrupted to allow the adsorbent to settle, and the elution was performed with 0.5*M* NaOH in a packed-bed mode. The contents of yeast cells and BA in the effluent during the adsorption, washing, and elution stages were assayed by measurement of the absorbance at 600 nm and after centrifugation at 270 nm, respectively.

### Adsorption of LA by f-cryoPVAG beads in an expanded-bed mode

Beads of f-cryoPVAG (4-mL settled volume) were put into a glass column (inner diameter = 10 mm) and washed in an expanded-bed mode at a linear flow rate of 160 cm/h with 10 bed volumes of 0.1*M* HCl followed by deionized water until the pH was close to 7.0. Five milliliters of sodium lactate solution (0.86 mg/mL), without cells and with 0.2 g (wet weight) of suspended yeast cells, was applied to the column at the same flow rate followed by washing with deionized water. The elution was performed in a packed-bed mode at a linear flow rate of 80 cm/h with 1*M* HCl. The cell content was assayed by measurement of the absorbance at 620 nm, and the LA content was assayed by HPLC after filtration of the samples with a 0.2-$\mu$m filter as described later.

### Adsorption of LA from a nonclarified fermentation broth by f-cryoPVAG beads in an expanded-bed mode

Beads of f-cryoPVAG (4-mL settled volume) were put into a glass column (inner diameter = 10 mm) and washed in an expanded-bed mode at a linear flow rate of 160 cm/h with 10 bed volumes of 0.1*M* HCl followed by deionized water until the pH was close to 7.0. The flow rate was adjusted to 160 cm/h to reach the twofold bed expansion when the nonclarified *Lactobacillus delbrueckii* fermentation broth was passed through the column. The nonclarified fermentation broth (2 mL) was applied at this flow rate followed by washing with deionized water. Elution was performed in a packed-bed mode with 1.0*M* HCl at a linear flow rate of 80 cm/h. The cell content during the adsorption, washing, and elution stages was assayed by measurement of the absorbance at 620 nm, and the LA content was assayed by HPLC as described later.

### LA determination

LA concentration was determined by HPLC (Shimadzu LC-6A, Kyoto, Japan) with an Aminex HPX-87H organic acid analysis column (300 $\times$ 7.8 mm, Bio-Rad, Hercules, CA) connected to an ultraviolet detector, column oven, and autosampler. The injection volume of the sample was 50 $\mu$L. The column temperature was maintained at 35°C. The elution was performed with 5 m*M* sulfuric acid at a flow rate 0.4 mL/min for 20 min, and the elution of LA was monitored by ultraviolet measurement at 220 nm. The retention time of LA under these conditions was 18.9 min. The column was preliminary standardized with Bio-Rad Organic Acid Standard.

### Preparation of samples for scanning electron microscopy

The samples of f-cryoPVAG were fixed in 2.5% glutaraldehyde at pH 1.0 for 4 h and then postfixed in 1% osmium tetraoxide for 1 h. Then, the samples were dehydrated in ethanol (0, 50, 75, and 99.5%) and critical-point-dried. The dried sample was coated with gold/palladium alloy (40/60) and examined with a JSM-5600LV scanning electron microscope (Jeol Ltd., Tokyo, Japan).

## RESULTS AND DISCUSSION

### Mechanical properties of composite cryoPVAGs

The commercial Amberlite resin in our disposal was in the form of beads approximately 500 $\mu$m in size, so the resin was ground to obtain smaller particles, and the fraction with a particle size of 25–100 $\mu$m was used as the filler for the composite cryogels. Ground Amber-

**TABLE I**
**Characteristics of the $OH^-$ and $Cl^-$ Forms of the Amberlite IRA-410 Resin Used as Fillers for the Preparation of f-CryoPVAGs**

| Counterion of the anion exchange resin | Particle size ($\mu$m)[a] | Total capacity of the anion exchange resin (mequiv/g of dry polymer)[b] | Swelling degree (g of water/g of dry polymer) | Content of dry matter in swollen resin (wt %) |
|---|---|---|---|---|
| $Cl^-$ | 25–100 | 3.4 | 0.9 | 52.8 |
| $OH^-$ | | | 2.1 | 31.7 |

[a] Measured with an optical microscope.
[b] Data from the Amberlite manufacturer.

lite was entrapped into composite in either hydroxide form ($OH^-$ form) or chloride form ($Cl^-$ form). The characteristics of the filler are summarized in Table I. The swelling degree of an anion-exchange resin depended on the nature of its counterions. The amount of ionic groups incorporated into cryogels with Amberlite was different when the $OH^-$ or $Cl^-$ forms were used as fillers at the same volume fraction ($\phi$). In Table II, the weight concentrations are presented along with the $\phi$ values of filler added into the composite.

The effect of the filler on the mechanical properties of f-cryoPVAGs is presented in Table II as the ratio of $G_0^f$ to $G_0$ of a nonfilled cryogel obtained under the same conditions and with the same concentration of PVA in a continuous phase. The f-cryoPVAGs with entrapped Amberlite particles in $Cl^-$ form were stronger than the corresponding cryoPVAGs. However, the incorporation of increasing amounts of the Amberlite in $Cl^-$ form had no significant reinforcing effect. In particular, 20 wt % ($\phi$ = 0.174) of the filler increased the f-cryoPVAG shear modulus only 1.4-fold compared to a nonfilled cryogel. In contrast, the entrapment of Amberlite in $OH^-$ form reinforced f-cryoPVAGs much more strongly (2.4–3.3 fold) and in a content-dependent manner (Table II). At the same $\phi$ of filler, the weight content of the disperse phase entrapped in the cryogel matrix was less in the case of adding resin in the $OH^-$ form than in the case of adding resin in the $Cl^-$ form (Table I). Thus, the significant reinforcement of f-cryoPVAGs with increasing amounts of resin in the $OH^-$ form could have been associated with the nature of the counterions of Amberlite rather than with the amount of filler added.

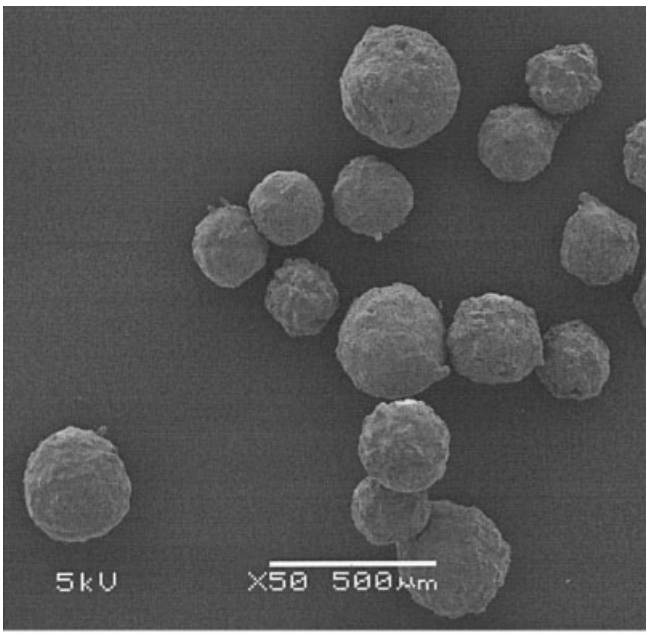
At the same time, it was found earlier[14] that the increasing concentration of hydroxyl ions in a starting PVA solution (without any fillers) weakened cryoPVAGs formed from such PVA solutions. Consequently, the significant reinforcement of a composite containing entrapped particles of a strong anion-exchange resin in $OH^-$ form was not due to the presence of hydroxyl ions themselves introduced into the gel matrix together with the disperse phase. Therefore, the reinforcement could have been related to conceivable additional interactions between the continuous and disperse phases caused by the presence of highly basic quaternary tetraalkylammonium groups at the resin surface. Additional interactions improve the adhesion of the polymer continuous phase to the filler surface and, as it is known,[9,23] increase the strength of composites in general.

A plausible rationale behind this effect in the case of cryoPVAGs containing entrapped particles of the anion-exchange resin in $OH^-$ form could be as follows.

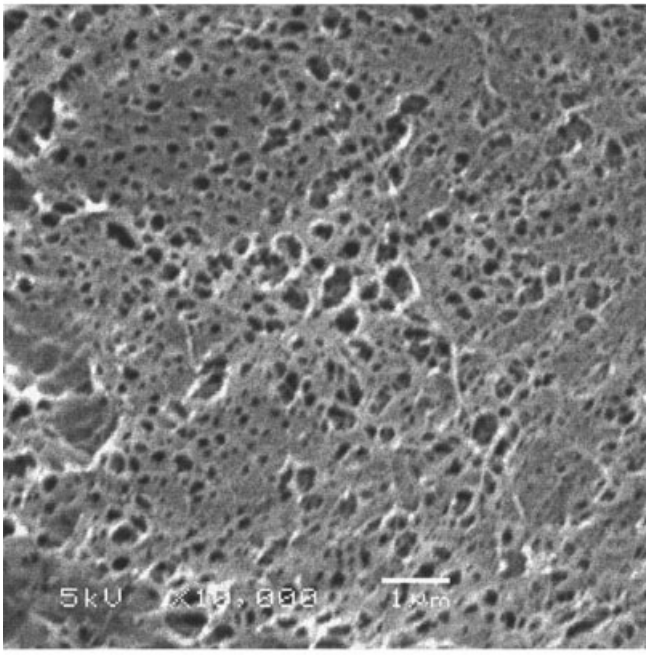
Under moderate (not deep) freezing conditions of suspension of the anion-exchange resin in PVA solution as the crystallization of ice proceeded, the local

**TABLE II**
**Effect of the Amberlite IRA-410 Filler Concentration and the Nature of the Counterion on the Mechanical and Thermal Properties of f-CryoPVAGs**

| Counterion of the anion exchange resin | Concentration of the swollen filler in the composite PVA cryogel (wt %) | $\varphi$ of filler | Concentration of dry matter of the filler in the composite PVA cryogel (wt %) | $G_0^f/G_0$ | $T_f$ |
|---|---|---|---|---|---|
| — | 0 | 0 | — | 1 | 68.2 ± 0.5 |
| $Cl^-$ | 5 | 0.044 | 2.64 | 1.13 ± 0.19 | 68.2 ± 0.3 |
| $OH^-$ | | 0.046 | 1.69 | 2.40 ± 0.29 | 73.9 ± 0.2 |
| $Cl^-$ | 10 | 0.087 | 5.28 | 1.36 ± 0.15 | 68.0 ± 0.3 |
| $OH^-$ | | 0.091 | 3.17 | 2.73 ± 0.24 | 74.0 ± 0.4 |
| $Cl^-$ | 15 | 0.130 | 7.92 | 1.42 ± 0.18 | 68.1 ± 0.6 |
| $OH^-$ | | 0.136 | 4.76 | 3.20 ± 0.53 | 74.4 ± 0.1 |
| $CI^-$ | 20 | 0.174 | 10.6 | 1.39 ± 0.15 | 68.4 ± 0.5 |
| $OH^-$ | | 0.182 | 6.34 | 3.33 ± 0.43 | 74.9 ± 0.1 |




(a)




(b)

**Figure 2** Scanning electron microscopy photographs of (a) f-cryoPVAG beads and (b) the surface of a bead at the magnification indicated by the bars at the bottom of the photographs. The samples were fixed in 2.5% glutaraldehyde at pH 1.0 for 4 h and were then postfixed in 1% osmium tetraoxide for 1 h, dehydrated in ethanol, and critical-point-dried (see Methods). Dried samples were coated with gold/palladium and examined with a Jeol JSM-5600LV scanning electron microscope.

increase in the concentration of $OH^-$ ions took place in the yet nonfrozen regions, thus resulting in a significant pH increase close to the surface of the filler particles. It is well known that PVA is partially ionized in alkali concentrations, that is, about 1% (0.15–0.2*M*) NaOH.[24] Thus, at high pH values, the deprotonation of the OH groups of PVA macromolecules and the formation of ionic bonds between the filler and gel matrix, such as $—N(C_2H_5)_3^+ \ldots O^-$, might have occurred. These ionic pairs were fixed after gel formation as the polymer chains lost their mobility, when the polymeric network was formed. After thawing, this interactions did not disappear because of the dilution with melting water, as happens in the case of an ordinary polymer solution when the gel network has not been formed in the frozen state. We believe that the combination of these physical and chemical events (the cryoconcentration of the $OH^-$ counterions of Amberlite-carrying tetraalkylammonium groups, the ionization of PVA hydroxyl groups, and the formation of ionic bonds followed by their subsequent fixation in formed cryogel) accounted for the more significant reinforcement of f-cryoPVAGs containing the anion-exchange resin in $OH^-$ form as compared to the reinforcement by the anion exchanger in $Cl^-$ form.

### Thermal properties of the composite cryoPVAGs

Similar to the mechanical properties, the thermal characteristics of the composite cryoPVAGs (Table II, last right column) also depended on the form of Amberlite entrapped into the cryogel matrix. The anion-exchanger filler in $Cl^-$ form virtually did not affect the $T_f$ of the composite. However, the addition of only 5 wt % ($\phi = 0.046$) of Amberlite in the $OH^-$ form increased $T_f$ by 5°C (note, to reach such an increase for a non-

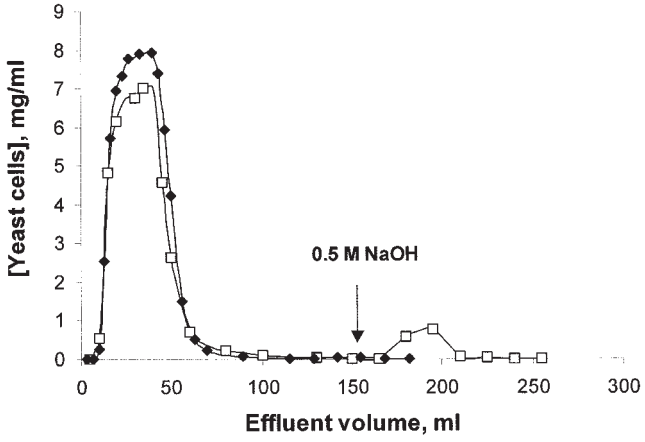



**Figure 3** Adsorption of yeast cells by (♦) f-cryoPVAG beads and (□) Amberlite resin. Experimental conditions: 5-mL settled bed volume in a 20.0 × 1-cm column at room temperature. The suspension of yeast cells (30 mL, 10 mg/mL) was passed through the column equilibrated with deionized water in an upward mode at flow rates of 260 and 764 cm/h for f-cryoPVAG beads and Amberlite resin, respectively. The elution was carried out with 0.5*M* NaOH in an expanded-bed mode.

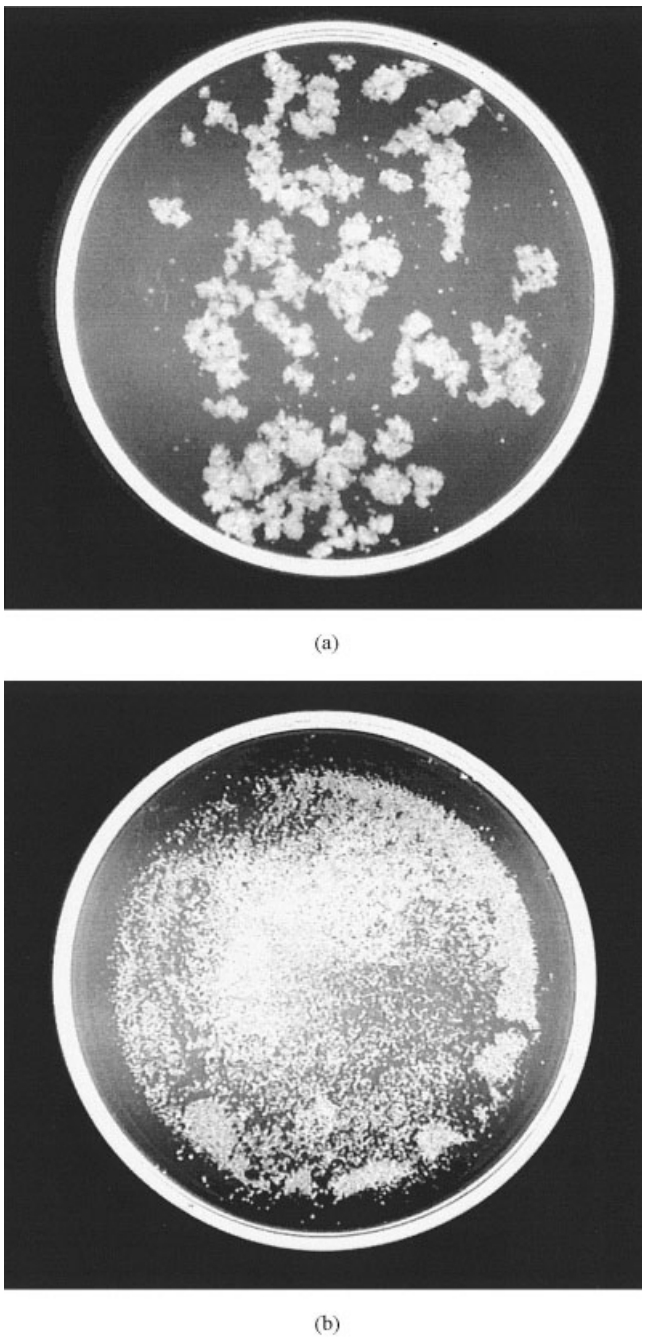


**Figure 4** (a) Amberlite resin beads and (b) f-cryoPVAG beads after contact with yeast cells.

filled cryoPVAG, the PVA concentration in the starting solution should be increased as much as 1.5–2.0 fold[25–28]). At the same time, the further increase in the amount of resin (in $OH^-$ form) to 20 wt % ($\phi = 0.182$) resulted only in a small incremental increase in $T_f$ of only about 1°C (Table II).

Because the falling-bead method used for $T_f$ determination evaluated only the melting of the gel matrix of f-cryoPVAGs (i.e., sol–gel conversion of thermoreversible phase of composite gel), the increase in $T_f$ caused by the entrapment of the anion exchanger in $OH^-$ form indicated that some changes did happen in the continuous gel phase itself. However, it is not yet clear why the formation of ionic bonds between the surfaces of disperse particles and PVA within a thin interphase layer could influence the polymer formation of a continuous gel phase in bulk, that is, far (at distances larger than size of several PVA macromolecules) from the particles.

### Direct capture of anionic solutes from a model fermentation broth with the composite cryogel

Composite adsorbent for the direct capture of anionic solutes from the model fermentation broth was prepared with a cryogranulation setup (Fig. 1). This method enabled us to prepare spherical beads of different sizes [Fig. 2(a)] by the pressing of the aqueous PVA solution through a liquid-jet head into the column filled with water-immiscible solvent (petroleum ether). The jet was splintered into droplets by the flow of petroleum ether. In the organic solvent, drops adopted a spherical form because of surface tension and froze falling down into the bottom of the column, where they were harvested in the collector. The droplets size, and so bead size, both depended on the flow rates of the aqueous and organic phases, nozzle diameter, and viscosity of the polymer solution. Through

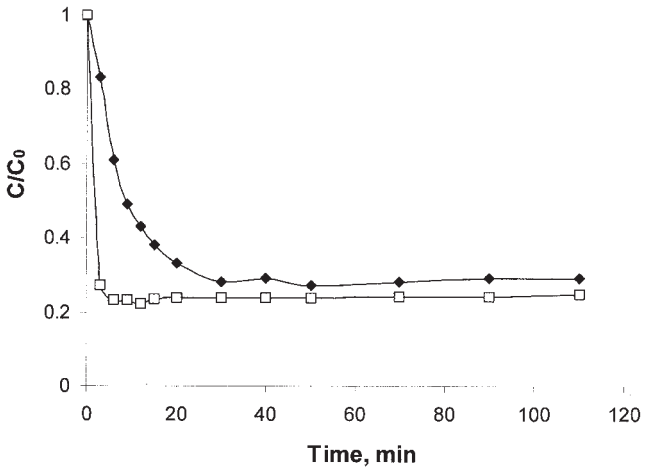



**Figure 5** Batch adsorption of BA by (□) f-cryoPVAG beads and (♦) Amberlite resin. Experimental conditions: 1.5 mL of adsorbent suspended in 1.5 mL of deionized water was incubated with 60 mL of BA solution (1 mg/mL, pH 7.0). The adsorption experiment was done at room temperature under constant stirring. Samples of the suspension (0.8 mL) were quickly removed at defined time intervals and centrifuged for 3 min at 6000 × g. The absorbance of supernatant was measured at 270 nm. The data are presented as the amount of BA adsorbed by 1 g of the less anion-exchange resin. $C/C_o$ is the relative concentration of BA in the breakthrough, where $C$ is the instant concentration and $C_o$ is the initial concentration.

**TABLE III**
**Adsorption of Benzoic Acid on a Column with Fluidized f-CryoPVAG Beads**

| Concentration of yeast cells (mg/mL) | Yeast cells in flowthrough and washings (wt %) | Benzoic acid in breakthrough (wt %) | Eluted benzoic acid with 0.5*M* NaOH (wt %) |
|---|---|---|---|
| 0 | 0 | 0.9 ± 0.2 | 99 ± 5 |
| 10 | 100 ± 9 | 3.0 ± 0.5 | 97 ± 5 |
| 10 | 100 ± 2 | 4.0 ± 0.2 | 96 ± 2 |
| 10 | 100 ± 18 | 4.7 ± 0.3 | 95 ± 4 |
| 20 | 100 ± 14 | 7.0 ± 0.5 | 93 ± 5 |
| 40 | 100 ± 13 | 17 ± 0.3 | 83 ± 5 |

the variation of these parameters, beads from 0.2 to 3 mm in size were obtained. It was important that drops did not freeze immediately and had time to adopt the spherical shape. Therefore, there was a temperature gradient in the column, and the organic solvent was warmer on the top and cooler toward the bottom.

In our case, spherical beads 200–600 $\mu$m in size were prepared, which were similar to the size of the commercial Amberlite used. Inside the f-cryoPVAG beads, the Amberlite particles were covered with a layer of cryoPVAG. The later was highly porous, as can be seen in Figure 2(b), with a pore size in the range of 0.1–1.0 $\mu$m. As the yeast cells were larger than 6 $\mu$m, they were unable to enter the pores. Practically no cells adsorbed to the f-cryoPVAG beads when a pulse of cells (10 mg/mL, 30 mL) was passed through the column in an expanded-bed mode at a flow rate of 260 cm/h. The consecutive elution with 0.5*M* NaOH generated no cells in the effluent.

In contrast, when the same pulse of yeast cells was applied to the fluidized Amberlite beads, about 5% of the applied cells were retained in the column. Some bound cells were eluted with 0.5*M* NaOH (3%), but 2% of bound cells were retained by the Amberlite resin practically irreversibly (Fig. 3). Despite the small amount of cells adsorbed to the Amberlite beads, the behavior of the beads changed dramatically. The Amberlite beads with bound cells aggregated strongly [Fig. 4(a)], compromising the stability of the expanded bed. At the same time, there were no any visible changes in the appearance of f-cryoPVAG beads after contact with the yeast cells [Fig. 4(b)].

Thus, the cryogel matrix prevented the adsorption of cells by the anion exchanger; however, the adsorption of a model low-MW substance, BA, was not impaired (Fig. 5). The macroporous structure of cryogels provided nonhindered diffusion of BA into the cryogel matrix and bounding to the anion-exchange resin entrapped there.

Therefore, direct capture of BA from the yeast cell suspension (a model fermentation broth containing both the negatively charged cells and the negatively charged target solute) was possible (Table III). The recovery of target product from the cell suspension was as high as 95–97%. Repeated cycles of BA binding elution on the column with composite adsorbent demonstrated a good reproducibility of column behavior (Fig. 6 and Table III). However, the increase in yeast cell content in the applied suspension somewhat decreased the efficiency of BA capture by the f-cryoPVAG beads (Table III). For instance, at cell contents of 10, 20, and 40 mg/mL in the applied solution, the amount of BA in the breakthrough (noncaptured by the column) was 4, 7, and 17%, respectively. The decreasing adsorbent capacity at high cell concentrations is a common phenomenon.[29,30] Authors have accounted for it by the undesirable interaction of cells with anion exchangers. Because yeast cells did not

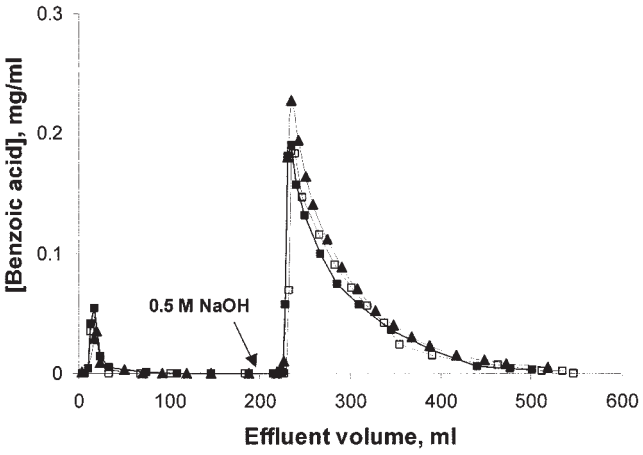



**Figure 6** Capture of BA by f-cryoPVAG beads in an expanded-bed mode from suspension with yeast cells. Experimental conditions: 5 mL suspension of 10 mg/mL yeast cells containing 2 mg/mL BA (pH 7.0) was applied to the column with f-cryoPVAG beads (5-mL settled volume) in an expanded-bed mode at a linear flow rate of 260 cm/h. The column was washed with deionized water in an expanded-bed mode. The flow was interrupted to allow the adsorbent to settle, and the elution was performed with 0.5*M* NaOH in a packed-bed mode at a linear flow rate of 80 cm/h. The content of yeast cells and BA in the effluent during the adsorption, washing, and elution stages was analyzed by measurement of the absorbance at 600 nm and after centrifugation at 270 nm, respectively. Different symbols on the elution curves show the values registered in different runs.

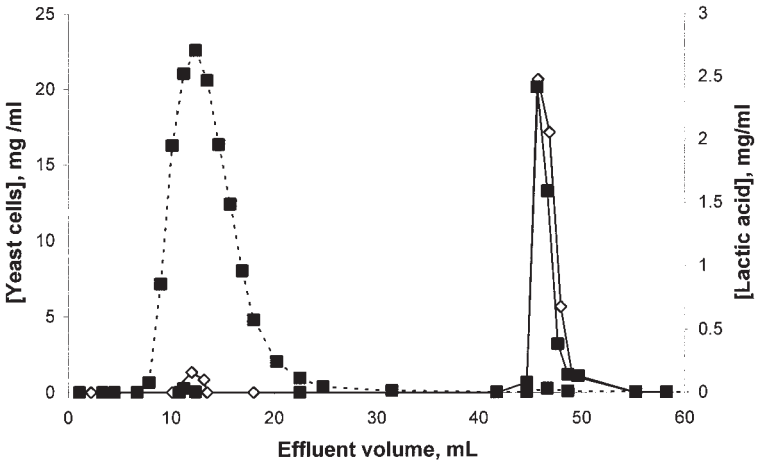



**Figure 7** Capture of LA by f-cryoPVAG beads in an expanded-bed mode. Experimental conditions: LA solutions without and with suspended yeast cells were applied to the column with f-cryoPVAG beads (4-mL settled volume) in an expanded-bed mode at a linear flow rate of 160 cm/h. The column was washed with deionized water in an expanded-bed mode. The flow was interrupted to allow the adsorbent to settle, and the elution was performed with 1.0*M* HCl in a packed-bed mode. The content of yeast cells was analyzed by measurement of the absorbance at 600 nm in the effluent during the adsorption, washing, and elution stages. LA was analyzed with HPLC. The dotted line and closed squares represent the cell content. The closed squares represent the LA concentration when the solution of LA in the suspension of cells was applied. The open diamonds represent the LA concentration during the sorption from the solution without cells.

adsorb on the f-cryoPVAG beads, the decreased BA binding in our case could obviously be explained by the adsorption of some competing impurities present in the cell suspension.

Analogously, LA could be efficiently captured in an expanded-bed mode from the 40 mg/mL yeast suspension (Fig. 7). Again, the presence of yeast cells decreased by about 20% the binding of LA acid as compared to the clear solution. This effect was much more pronounced when the nonclarified *Lactobacillus delbrueckii* fermentation broth was directly applied on an expanded-bed column. The binding of LA dropped to only 40% of the original, resulting in the presence of a large amount of LA in the breakthrough fraction (Fig. 8). Nevertheless, the bound LA was recovered with 96% yield. Probably, the drastically reduced binding of LA was due to the presence of other anionic low-MW contaminants in the fermentation liquid. These contaminants could have efficiently competed with LA for binding to the anion exchanger.

## CONCLUSIONS

A new type of composite material was produced. It was composed of a cryoPVAG with entrapped particles of the strong anion-exchange resin Amberlite. The effect of entrapment of particles into cryoPVAGs on the properties of the composite material depended strongly on whether the resin was used in the $OH^-$ form or $Cl^-$ form. Beads 200–600 $\mu$m in size were prepared from the composite material and used in expanded-bed ion-exchange chromatography for the capture of negatively charged solutes, benzoate, and lactate from the suspension of negatively charged cells. The plausibility of the approach was demonstrated on model systems composed of yeast cells and

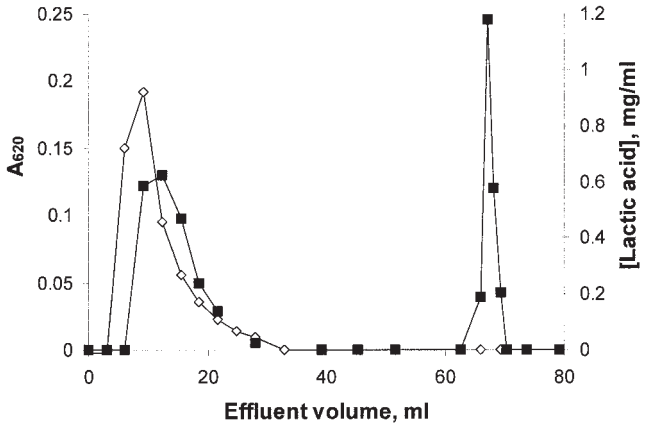



**Figure 8** Capture of LA by f-cryoPVAG beads in expanded-bed mode from nonclarified *Lactobacillus delbrueckii* fermentation broth. Experimental conditions: 2 mL of nonclarified broth was applied to the column with f-cryoPVAG beads (4-mL settled volume) in an expanded-bed mode at a linear flow rate of 160 cm/h. The column was washed with deionized water in an expanded-bed mode. The flow was interrupted to allow the adsorbent to settle, and the elution was performed with 0.1*M* HCl in a packed-bed mode. The content of cells was analyzed by measurement of the absorbance at 620 nm in the effluent during the adsorption, washing, and elution stages. LA was analyzed with HPLC: (♦) cell content and (■) LA concentration. $A_{620}$ is the light absorbance at 620 nm.

benzoate and with a real fermentation broth produced after LA fermentation. The use of composite adsorbents offered the advantage of the maintenance of the adsorbent capacity but in a protected form such that interactions with particulate matter were prevented. This leads to the possibility of also using the adsorbent in complex mixtures. However, in such cases it might be desirable to use adsorbents with higher selectivities. The study presented here clearly documented that the new composite materials might be of interest for dealing with the isolation of solutes from complex suspensions.

## References


1. Nambu, M. Kobunshi Ronbunshu 1990, 47, 695 (in Japanese).
2. Peppas, N. A.; Stauffer, S. R. J Controlled Release 1991, 16, 305.
3. Lozinsky, V. I. Russ Chem Rev Eng Ed 1998, 67, 573.
4. Hassan, C. M.; Peppas, N. A. Adv Polym Sci 2000, 153, 37.
5. Lazzeri, L. Trends Polym Sci 1996, 4, 249.
6. Lozinsky, V. I.; Plieva, F. M.; Galaev, I. Y.; Mattiasson, B. Bioseparation 2001, 10, 163.
7. Lozinsky, V. I. Russ Chem Rev Engl Ed 2002, 71, 489.
8. Lozinsky, V. I.; Galaev, I. Y.; Plieva, F. M.; Savina, I. N.; Jungvid, H.; Mattiasson, B. Trends Biotechnol 2003, 21, 445.
9. Lozinsky, V. I.; Zubov, A. L.; Kulakova, V. K.; Titova, E. F.; Rogozhin, S. V. J Appl Polym Sci 1992, 44, 1423.
10. Lozinsky, V. I.; Zubov, A. L.; Titova, E. F. Enzyme Microb Technol 1997, 20, 182.
11. Lozinsky, V. I.; Damshkaln, L. G. J Appl Polym Sci 2001, 82, 1609.
12. Lozinsky, V. I.; Savina, I. N. Colloid J 2002, 64, 336.
13. Lozinsky, V. I.; Domotenko, L. V.; Vainernan, E. S.; Mamtsis, A. M.; Titova, E. F.; Belavtseva, E. M.; Rogozhin, S. V. Colloid Polym Sci 1986, 264, 19.
14. Lozinsky, V. I.; Zubov, A. L.; Titova, E. F. Enzyme Microb Technol 1996, 18, 561.
15. Shaheen, S. M.; Yamaura, K. J Controlled Release 2002, 81, 367.
16. Chase, H. A. Trends Biotechnol 1994, 12, 296.
17. Feuser, J.; Walter, J.; Kula, M.-R.; Thommes, J. Bioseparation 1999, 8, 99.
18. Willcox, P. J.; Howie, D. W.; Schimdt-Rohr, K.; Hoagland, D. A.; Gido, S. P.; Pudjijanto, S.; Kleiner, L. W.; Venkatraman, S. J Polym Sci Part B: Polym Phys 1999, 37, 3438.
19. Fergg, F.; Keil, F. J.; Quader, H. Colloid Polym Sci 2001, 279, 61.
20. Szczesna-Antczak, M.; Galas, E. Biomol Eng 2001, 17, 55.
21. Lozinsky, V. I.; Zubov, A. L. Russ. Pat. No. 2036095 (1992).
22. Hrouz, J.; Ilavsky, M.; Havlicek, J.; Dusek, K. Collect Czech Chem Commun 1978, 43, 1999.
23. Lipatov, Y. S. Adv Polym Sci 1977, 22, 1.
24. Ushakov, S. N. Poly(vinyl alcohol) and Its Derivatives; USSR Academy of Sciences: Moscow, 1960; Vol. 1, p 297 (in Russian).
25. Domotenko, L. V.; Lozinsky, V. I.; Vainerman, E. S.; Rogozhin, S. V. Vysokomol Soedin A 1988, 30, 1661 (in Russian).
26. Watase, M.; Nishinari, K. Makromol Chem 1989, 190, 155.
27. Lozinsky, V. I.; Domotenko, L. V.; Zubov, A. L.; Simenel, I. A. J Appl Polym Sci 1996, 61, 1991.
28. Lozinsky, V. I.; Zubov, A. L.; Savina, I. N.; Plieva, F. M. J Appl Polym Sci 2000, 77, 1822.
29. Chase, H. A.; Drager, N. M. J Chromatogr 1992, 597, 129.
30. Mullick, A.; Flickinger, M. C. Biotechnol Bioeng 1999, 65, 282.